REMARKS

The specification has been amended to incorporate the SEQ ID NOS of the various sequences disclosed therein by their respective SEQ ID NOS as assigned in the Sequence Listing being submitted concurrently herewith. No new matter is introduced by virtue of these amendments. Accordingly, Applicants kindly request that they be entered into the instant application.

The Commissioner is hereby authorized to charge any required fee or credit any overpayment to Pennie & Edmonds LLP Deposit Account no. 16-1150. This page is submitted in duplicate for such purpose.

Respectfully submitted,

Dated: August 21, 2001

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Exhibit A
Marked Up Versions of Amended Paragraphs
(Additions are italicized, deletions are bracketed)

Amended paragraph on page 8, beginning, "The plasmids pHOG-αCD19 and pHOG-dmOKT3 which code for the scFv fragments derived from the hybridoma...":

The plasmids pHOG-αCD19 and pHOG-dmOKT3 which code for the scFv fragments derived from the hybridoma HD37 which is specific to human CD19 (Kipriyanov *et al.*, 1996, *J.-Immunol. Meth.* 196:51-62) and from the hybridoma OKT3 which is specific to human CD3 (Kipriyanov *et al.*, 1997, *Protein Eng.* 10:445-453), respectively, were used for the construction of expression plasmids for a single-chain F_v antibody construct. A PCR fragment 1 of the V_H domain of anti-CD19, followed by a segment which codes for a GlyGly linker, was produced using the primers DP1, 5'-TCACACAGAATTC-TTAGATCTATTAAAGAGGAGAAATTAACC *(SEQ ID NO:1)* and DP2, 5'-AGCACACGATATCACCGCCAAGCTTGGGTGTTGTTTTGGC *(SEQ ID NO:2)* FIGURE. 2). The PCR fragment 1 was cleaved by EcoRI and EcoRV and ligated with the EcoRI/EcoRV-linearized plasmid pHOG-dmOKT3 so as to produce the vector pHOG19-3. The PCR fragment 2 of the V_L domain of anti-CD19, followed by a segment which codes for a c-myc epitope and a hexahistidinyl tail, was produced using the primers DP3, 5'-AGCACACAAGCTTGGCGGTGATATCTTGCTCACCCAAAC-TCCA *(SEQ ID NO:3)* and DP4,

5'-AGCACACTCTAGAGACACACAGATCTTTAGTGATGGTGAT-GGTGATGTGAGTT TAGG *(SEQ ID NO:4)*. The PCR fragment 2 was cleaved by HindIII and XbaI and ligated with the HindIII/XbaI-linearized plasmid pHOG-dmOKT3 so as to obtain the vector pHOG3-19 (FIGURE 2). The gene coding for the hybrid scFv-3-19 in the plasmid pHOG3-19 was amplified by means of PCR with the primers Bi3sk,

5'-CAGCCGGCCATGGCGCAGGTGCAACTGCAGCAG *(SEQ ID NO:5)* and either Li-1,

5'-TATATACTGCAGCTGCACCTGGCTACCACCACCACCGGAGCCG-CCACCACC GCTACCACCGCCGCCAGAACCACCACCACCAGCGGCCGCAGCATCAGCCCG *(SEQ ID NO:6)* for the production of a long flexible $(Gly_4Ser)_4$ inter-scFV linker (PCR fragment 3, FIGURE 2) or Li-2,

5'-TATATA-CTGCAGCTGCACCTGCGACCCTGGGCCACCAGCGGCCGCAGCATC

AGCCG, *(SEQ ID NO:7)* for the production of a short rigid GGPGS linker (PCR fragment 4, FIGURE 2). The expression plasmids pDISC3x19-LL and pDISC3x19-SL were constructed by ligating the NcoI/PvuII restriction fragment from pHOG19-3, comprising the vector framework and the NcoI/PVuII-cleaved PCR fragments 3 and 4, respectively (FIGURES 3 and 4). The complete nucleotide and protein sequences of the bivalent and tetravalent F_v antibody constructs are indicated in FIGURES 5 and 6, respectively.

Amended paragraph on page 9, beginning, "The vector pPICZαA (Invitrogen BV, Leek, Netherlands) for the expression and secretion of recombinant proteins..."

The vector pPICZαA (Invitrogen BV, Leek, Netherlands) for the expression and secretion of recombinant proteins in the yeast *Pichia pastoris* was used as a starting material. It contains a gene which codes for the *Saccharomyces cerevisiae* α-factor secretion signal, followed by a polylinker. The secretion of this vector is based on the dominant selectable marker, Zeocin™ which is bifunctional in both *Pichia* and *E. coli*. The gene which codes for the tetravalent F_v antibody construct (scDia-SL) was amplified by means of PCR by the template pDIC3x19-SL using the primers 5-PIC, 5'-CCGTGAATTCCAGGTGCAACTGCAGCAGTCTGGGGCTGAACTGGC, and pSEXBn *(SEQ ID NO:8)*. 5'-GGTCGACGTTAACCGACAAACAACAGATAAAACG *(SEQ ID NO:9)*. The resulting PCR product was cleaved by EcoRI and XbaI and ligated in EcoRI/XbaI-linearized pPICZαA. The expression plasmid pPIC-DISC-SL was obtained. The nucleotide and protein sequences of the tetravalent F_v antibody construct are shown in FIGURE 7.

Amended paragraph on page 13, beginning, "Expression vectors were prepared which contained the hok/sok plasmid-free cell..."

Expression vectors were prepared which contained the hok/sok plasmid-free cell suicide system and a gene which codes for the skp/OmpH periplasmic factor for a greater production of recombinant antibodies. The skp gene was amplified by PCR using the primers skp-1, 5'-CGA ATT CTT AAG ATA AGA AGG AGT TTA TTG TGA AAA AGT GGT TAT TAG CTG CAG G *(SEQ ID NO:10)* and skp-2, 5'-CGA ATT AAG CTT CAT TAT TTA ACC TGT TTC AGT ACG TCG G *(SEQ ID NO:11)* using the plasmid pGAH317 (Holck and Kleppe, 1988, *Gene* 67:117-124). The resulting PCR fragment was cleaved by

AflII and HindIII and inserted in the AflII/HindIII-linearized plasmid pHKK (Horn et al., 1996, Appl. Microbiol. Biotechnol. 46, 524-532) so as to obtain the vector pSKK. The genes obtained in the plasmids pDISC3x19-LL and pDISC3x19-SL and coding for the scFv antibody constructs were amplified by means of the primers fe-1, 5'-CGA ATT TCT AGA TAA GAA GGA GAA ATT AAC CAT GAA ATA CC *(SEQ ID NO:12)* and fe-2, 5'-CGA ATT CTT AAG CTA TTA GTG ATG GTG ATG GTG ATG TGA G *(SEQ ID NO:13)*. The XbaI/AflII-cleaved PCR fragments were inserted in pSKK before the skp insert so as to obtain the expression plasmids pDISC5-LL and pDISC6-SL, respectively, which contain tri-cistronic operons under the control of the lac promoter/operator system (FIGURES 9 and 10).